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Development of an Economic Large Scale Growth Medium for Rhizopus arrhizus, A Uranium Adsorbing Fungi

Final Report

for

DSS - Uranium Fungi
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Earth Resources Group
Science Branch
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Development of an Economic Large Scale Growth Medium for Rhizopus arrhizus for Department of Supply and Services

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EXECUTIVE SUMMARY

The objective of this study was to investigate the utilization of industrial food waste as a growth medium for the U-biosorbing fungus Rhizopus arrhizus. Associated with the above are biomass yields of 20-25 g/L growth medium, capable of U-uptake of 130 mg/g biomass. The testwork was carried out on a bench scale starting with 14 different food wastes ultimately being reduced to one; spent brewery grain. Optimization of growth parameters included agitation rates, medium strength and inoculum concentration. By harvesting biomass before the onset of the death phase, U-uptake values were maintained. Batch U-loading testwork carried out indicated optimal S:L ratios of 1:200 (95% U-removal) and 1:1000 with up to 62% U removal. Sodium carbonate proved to be an effective elution medium. Further work is recommended to integrate these findings with a proven immobilization scheme and be developed as a viable U-scavenging technique.

1. INTRODUCTION

Fungal biomass has been shown to be capable of removing uranium from solution and as such has the potential to become incorporated into a processing flow sheet for recovering uranium from dilute mine waters. In the development of this biotechnology, a number of unit processes must be economically minimized; among these the growing and harvesting of the biomass. Producing the biomass for uranium - adsorption has usually depended on a commercially available growth medium introducing the potential for increased processing costs. With the availability of a wide variety of industrial based food waste, ORTECH has investigated the possibility of maximizing the biomass harvest using food waste as the growth medium. This present program is utilizing the proven uranium-adsorbing fungus Rhizopus arrhizus in attempting to develop an economic alternative growth substrate that will support the maximum biomass harvest as well as maintain high uranium-adsorption values.

2. EXPERIMENTAL

The experimental work is divided into nine tasks:

Task 1	Compilation of industrial food waste available in Southern Ontario.
Task 2	Procurement of food waste
Task 3	Chemical (C, N, P) analysis
Task 4	Fungal maintenance
Task 5	Growth medium design and formulation

Task 6 Screening the growth of Rhizopus arrhizus in the various food waste preparations.

Task 7 Growth and morphological optimization

Task 8 Harvesting and processing biomass

Task 9 Uranium loading and elution tests

2.1 Compilation of Available Industrial Scale Food Waste

A total of thirty (30) different food producers were contacted as to the availability of waste associated with their operations. A list of those producers contacted and the results obtained appears in Table 1. Sources of information were the Ontario Manufacturers Directory and the Ontario Waste Exchange.

2.2 Procurement of Food Waste

Eight (8) industrial food producers ultimately agreed to supply fourteen (14) different forms of food-based waste, all of which could be obtained again if necessary. A brief description of the industrial source and the waste produced is presented in Table 2. The wastes were either shipped to ORTECH or picked up at the source. Following receipt of the waste products, they were sampled as received and stored frozen for further investigations.

2.3 Chemical Analysis

Measured volumes of distilled water were used to homogenize the alternate food wastes to provide representative samples for assaying. A description of the food wastes submitted for chemical analysis is found in Table 3. These were then assayed for Total Organic Carbon (TOC), Total Kjeldahl Nitrogen (TKN), both measured by Automated Colorimetry (A. Col), and Total Phosphorous (TP) measured by Inductively-Coupled Argon Plasma Spectroscopy (ICAP). The samples were numbered UF-01 to UF-16 and referred to by these numbers throughout the report. Two samples, UF-01 and UF-02 were submitted as UF-14 and UF-15 respectively to indicate reproducibility of results. The results of analysis are presented in Table 4.

2.4 Fungal Maintenance

A sample of Rhizopus arrhizus was received from Dr. R.G.L. McCready, CANMET. The culture sample and all subsequent manipulation were handled using aseptic technique. In the early stages of this investigation, the fungus was cultured on Potato Dextrose Agar (PDA) every 30 days minimum. Once the culture had developed sporangiophores, a petri dish bearing the so-grown culture was stored at 4°C. When the actual growth experiments in the food waste media were started, the culture was grown as required, usually once per week or more.

Preparation of PDA (Difco) was followed as per directions: completely dissolve 39 g/L distilled water before autoclaving; sterilize in autoclave for 15 minutes at 15 pounds pressure (121°C); cool to 45-50°C and dispense in Petri-dishes (15-20 mL/dish). To culture Rhizopus arrhizus, 0.1 mL of a spore

suspension was overlaid on the surface of the PDA and incubated at room temperature. Spore suspensions were prepared with 0.1 M phosphate buffer, pH 5.8, by agitating sporangiophores into a volume of buffer, then passing the suspension of mycelium and spores through sterilized cotton batten.

General methods in mycological studies were consulted from C. Booth (1971).

2.5 Growth Medium Design and Formulation

A theoretical maximum yield of 20-25 grams biomass per litre growth medium (4-5 g. dry weight) is what the formulation of these food waste media are based on. Non-growth-limiting concentrations of the three assayed essential nutrients, C, N, P, were used in all preparations. Critical assumptions utilized in medium formulation are:

1. 0.4-0.5 g, dry wt. cells per gram glucose
2. ≤ 13% N in cellular composition
3. ≤ 3% P in cellular composition
4. other essential and trace elements incorporated as part of the food waste and dilution tap water.

Initial medium formulations were prepared using these guidelines. However, following the preparation and subsequent inoculation and growth of these media, it was found that due to the "thick, fibrous" consistency of most of the media, biomass determinations were not feasible. Results, nevertheless, were obtained based on a visual assessment of mycelial growth and are presented in Table 5. Additional experiments were run to determine the effect of the stationary mode on cultures growing in five (5) of the raw

blended food wastes with results being presented in Table 6. Modifications to the initial plan were therefore made incorporating the preparation of extracts made with the food wastes. These extracts were made following procedures outlined by Calam (1969), with the assumption that the optimal C:N:P ratios for fungal growth would be present.

The raw blended food waste growth characteristics were used in the selection of food wastes to prepare extracts. Eight wastes were subsequently made into extracts with the preparation specifics presented in Table 7. Visual observations were made on preliminary experiments which compared diluted extract (final volume diluted to 1L) to full strength extract. These results are presented in Table 8 with subsequent gravimetric determinations of the biomass produced in each of the extracts being presented in Table 9.

2.6 Growth of Rhizopus arrhizus

Food waste extracts were inoculated with spore suspensions of Rhizopus arrhizus. Spore suspensions were prepared as in 2.4 with the spore inoculum concentration being kept constant at 2.5 x 10^5 spores/mL throughout this investigation using the cell counting method of the Neubauer Haemocytometer chamber. The formula used is:

$$\frac{\text{number of spore counted x dilution x 4000}}{\text{number of chamber squares counted}}$$

Food waste extracts were prepared in 100 mL aliquots in 500 mL Erlenmeyer flasks and cultured at 23°C, 50% relative humidity on an orbital shaker at 200 rpm.

Biomass yeilds were measured gravimetrically. The flask contents were filtered through three layers of preweighed cheesecloth then washed with 500 mL warm tap water to remove traces of the extract. The biomass and cheesecloth were then dried in vacuo at 40°C, allowed to equilibrate to room temperature and humidity following drying, and weighed to the nearest hundredth gram. Wet and dry cheesecloths were included in the drying to determine weight losses attributable to the cheesecloth alone.

The pH was monitored during growth experiments by immersing the pH electrode in the cheesecloth filtered culture medium and measuring to the nearest 0.01 pH unit.

To conduct a growth experiment, 5 to 6 flasks containing inoculum and extract were incubated for 50-100 hours. At each prearranged time interval, one flask and contents would be harvested as described in the above paragraph. Samples were frozen before drying.

2.7 Growth and Morphological Optimization

The inoculum spore concentration was optimized by varying the spore count as determined by a Neubauer Haemocytometer. Concentrations of 2.5×10^5 spores/mL and 2.5×10^6 spores/mL were inoculated into 100 mL volumes of full strength spent brew grain extract and agitated at 200 rpm. Growth and morphology of the developing fungus was monitored and reported on as visual observations.

Following optimization of the inoculum, the SBG medium strength and agitation rates were examined. Growth of the fungus, as determined gravimetrically, was monitored between 2 different SBG media strengths; full strength extract from 200 g SBG/L, and double strength extract from 400 g SBG/L. As well. both the above variables were examined for growth rates at different agitation speeds; 200 rpm and 300 rpm. The above results are presented in Figures 1-6.

2.8 Harvesting and Processing Biomass

Obtaining quantities of Rhizopus arrhizus biomass, large enough for bioadsorption experiments, required growing the fungus in larger volumes. For this task, 200 mL volumes of double strength medium in 1L Enlenmeyer flasks were incubated with 2mL of a $2.5x10^5$ spores/mL inoculum, and incubated at 25°C, 50% R.H. and agitated at 300 rpm. The pH was monitored so as to obtain biomass before the characteristic rise in pH during growth occured.

To harvest the biomass, the culture was filtered through 3 layers of cheesecloth, washed twice in 30 minute warm tap water washes, autoclaved at 12 psi for 20 minutes, and finally freeze-dried. The dried biomass was stored in sealed bottle with dessicant until used.

2.9 Loading and Elution Tests

Freeze dried biomass was comminuted with a mortar and pestle to a powdery consistency. The prepared biomass was then used in the bioadsorption assays (Figure 7-16).

As - received Denison mine water had a pH of 2.45 and required increasing to pH 4.0. This was accomplished with a saturated NaOH solution. Following pH adjustment, the heavily precipitated mine water was allowed to settle and further decanted and filtered through a 0.45 μ Millipore filtration unit.

The pH 4 mine water then was contacted in a range of solid to liquid ratios from between 1:200 to 1:2000 to obtain maximal U-uptake values. Flasks with biomass and mine water were agitated at 200 rpm on a Burell wrist action shaker. Uranium adsorption was monitored for 1 hour, taking samples at 5, 15, 30 and 60 minutes.

The samples were then 0.45 μ Millipore filtered and submitted for U-analysis (total U in solution) by DCP.

Elution of the loaded biomass was performed using 5% Na_2CO_3 in batch conditions. Flasks of biomass and carbonate at solid to liquid ratios of 1:1000 to 1:2000 were agitated on the Burell wrist action shaker at 200 rpm with samples taken 60 minutes. These were filtered as above and filtrate submitted for U-analysis by DCP.

3. RESULTS AND DISCUSSION

3.1 Compilation of Industrial food waste available in Southern Ontario

The results of a survey of industrial scale food producers and the wastes they produce is presented in Table 1. Of the thirty (30) representatives contacted, ten (10) produced no waste, five (5) produced wastes of a seasonal nature and were unavailable at the time of procurement, two (2) had bureaucratic complications, two (2) were undesirable, one (1) was produced in a distant city Thunder Bay, one (1) recycled their waste products, and one (1) landfilled their waste. Eight (8) represntatives agreed to supply a variety of waste as detailed in Table 2.
The Village of Hensall supplied 2 wastes, bean dirt and grain screenings, both appearing to be agricultural waste. The bean dirt was dry and looked to be composed of beans and soil. The grain screenings were dry and composed of bits of grain and straw.

Omstead Foods supplied (1) raw fish composed of parts and small waste fish, as well as (2) onions composed of waste onion peelings.

Nabisco Foods supplied dry, floor sweepings of cereal discards, specifically Shredded Wheat and Shreddies mixed.

Heritage Farms supplied 2 products. The vitamin beveridge was a homogeneous milky white liquid obtained as a result of large scale test work by Heritage, The pig slop was a random sampling of the waste normally produced by Heritage and stored in large containers waiting for removal to pig farms.

Campbell Mushroom Farms supplied waste mushrooms, picked up from fresh and partially frozen waste piles. A compost composed of dirt, mushrooms and mushroom growth medium was also donated.

Protein Foods supplied chicken waste water, a foul-smelling liquid with various cooked chicken bits floating on the surface.

Connors Brewery supplied spend brew grain which was the waste product generated by the wort preparation. There was various bits of kernals and sprouted grain in a slightly moist slurry.

3.2 Chemical Analysis

Results of chemical analysis for Total Kjeldahl Nitrogen (TKN), Total Organic Carbon (TOC) and Total Phosphorous (TP) of the as submitted and as recieved food waste are presented in Tables 3 and 4 respectively. The values in Table 3 detail the dilutions necessary to submit well blended samples for analysis. Table 4 represents the results of analysis of the food wastes blended and diluted as well as the converted values for the as-received food waste.

TKN values of the food wastes are generally >2000 ppm. Two samples, onions and chicken waste water, immediately stand out as having extremely low TKN values, 1140 ppm and 570 ppm respectively. Both these samples also show low values of TOC and TP, precluding any subsequent investigations. TKN values

are greatest in the bean dirt, cereal and fish wastes as are the TP values. TOC values are greatest in the potato chips, grain screenings, cereal, bean dirt and corn wastes. The high TOC values are present in wastes which have very low water content, except the corn.

3.3 Growth Medium Design and Formulation

Design of raw, blended food waste media preparations proceeded using cellular C, N, and P requirements as a guideline. Using bean dirt as an example, the following calculations and measurements were made:

(UF-1 and UF-14 both bean dirt samples)

TKN	$\bar{x}$ = 5.15 mg/g suspension (3 parts H_2O/1 part waste)
TOC	$\bar{x}$ = 7.55 g/100 g suspension (3/1)
TP	$\bar{x}$ = 0.75 mg/g suspension (3/1)

to incorporate:		medium requires:
≥ 125 g C/L	→	1655g of 3/1 suspension
≥ 320 mg N/L	→	62g of 3/1 suspension
≥ 60 mg P/L	→	80g of 3/1 suspension

One liter of bean dirt suspension will weigh approximately 1000g, therefore a further calculation reveals that a 3/1.58 H_2O/bean dirt suspension would provide this.

In designing this growth medium using raw, blended food waste, the fibrous nature of the preparations was overlooked. Once the media had been cultured, it was realized that biomass gravimetric determinations would not be possible as the mycelium could not be separated from the food waste. With growth observed as being either good or marginally good, hot water extracts of these wastes were prepared (Table 7).

The hot water extracts of 8 of the waste samples provided for a clear, homogeneous although chemically undefined medium. Freezing the once-filtered extract produced a precipitate removable by filtration, and prevented precipitates occuring in autoclaved extracts.

Carbon, nitrogen and phosphorous values were implied from the amounts used in the extract preparation. As shown in Table 10, the 8 food wastes have a wide range of maximum theoretical TOC values, while the TKN and TP values cluster about similar values respectively. Specific gravity measurements were made to make comparisons with known sucrose solutions. Although sucrose probably does not exist in solution, soluble carbohydrate values may be implied. All the extracts theoretically have from between 10g/L to 23g sucrose/L, indicative of S.G. measurements of between 1.002 to 1.007.

With cell production about 50% efficient when grown on glucose (0.4-0.5 g cells/g glucose) it is not unreasonable to assume that whatever soluble carbohydrates are present in solution, if incorporated in excess, will allow for growth of the fungus.

3.4 Growth of Rhizopus arrhizus

In screening the raw blended food waste for growth potential, visual assessments of growth were made (Table 5). Growth seemed to occur only at the swirl line of the "thick" preparations, if any at all. No growth was observed in the vitamin beveridge or the pig slop, both obtained from Heritage Farms Dairy. A very thin ring of growth in the mushroom medium as well as the above observations finished any further investigations with these 3 wastes. The other food wastes, separate and combinations of, had either poor (fish/potato chips, chips/corn/vitamin beveridge/pig slop) or moderate to good growth. The poor or no growth may be explained as a result of preservatives and/or inhibitors present in the dairy beveridges and/or chips.

Stationary flasks (as compared to the shake-flask experiments above) displayed as good a growth as the shake flasks but was accompanied by sporulation, an undesirable characteristic with respect to biomass harvests (Table 6).

Extract preparations were at first visually assessed for growth, to determine if the fungus would indeed grow. Five extracts were prepared and either grown full strength or diluted to one liter strength. Results are reported on in Table 8 where the dilute preparations showed less growth than the full strength. Full strength media preparations were subsequently used in further investigations.

Gravimetric determinations were made for growth in the extracts, results of which are presented in Table 9. A number of extracts, either diluted to one liter or full strength, had growth evident but gravimetrically showed losses in mass. Bean dirt, corn and a mixture of fish and grain screens were assayed repetitively but failed to show any stability in biomass gains. The cereal and the spent brew grain (SBG) extracts tended to show consistent biomass gain and as such were chosen for further optimization.

Comparisons of growth in the cereal extract medium and the SBG medium were made to narrow the optimization task even further. Figures 1 and 2 show slightly faster growth rates for the cereal. The SBG extract on the other hand has biomass gains which are slightly higher. Based on these results, the SBG extract was investigated further.

The optimal spore concentration to use as inoculum was determined as 1 mL of a 2.5×10^5 spores/mL suspension. A higher spore concentration, 2.5×10^6 spores/mL (1mL) resulted in poor to no mycelial development with only slight development of the inoculum into the "pellet" growth habit.

Figure 3 and 4 show the effects of increasing the medium strength from single (FS) to double (DS) agitated at 200 rpm on a gyrotary shaker. Rhizopus arrhizus shows slightly faster growth in the DS medium (Figure 3), while in Figure 4 biomass gains have significantly increased from approximately 1.2 g dry wt./L medium (FS) to approximately 2.5 g dry wt./L at 40 hours. By increasing the agitation speed to 300 rpm (Figure 5), biomass gains decreased to approximately half in the FS medium while the DS medium showed a increase to 4-5 g dry wt./L medium. Figure 6 shows a confirmation of DS 300 results.

By achieving the 4-5 g/L of biomass, the double strength medium agitated at 300 rpm has been selected as the optimal medium.

3.5 Harvesting Biomass

The termination of growth was accompanied by a gradual decline of pH change. As well, the clump of biomass started to break apart and appear shredded. The pH continues to decline but does so at a slower rate than during the logarithmic phase of growth. At pH >3, the harvesting of the biomass would occur near the end of the log phase of growth and therefore present biomass still metabolically active. This idea was discussed by Treen-Sears et al. (1984), whereby U-uptake decreased with late harvested Rhizopus arrhizus biomass. Although the U-uptake of differently harvested biomass was not tested here, cells harvested in this late log phase did produce reasonable results.

Cell texture appeared to be of a clump of intertwined mycelium, which required cutting into workable sized pieces for efficient mortar and pestle comminution. The colour of the biomass was a very pale beige throughout the harvest, autoclave inactivation and freeze drying procedures.

Following biomass removal from the double strength SBG, specific gravity measurements revealed that a decrease from 1.005 to about 1.001 had occured. This would indicate the removal of the equivalent of approximately 18 g sucrose/L medium.

3.6 Loading and Elution Tests

In a range of solid to liquid (S:L) ratios between 1:200 and 1:2000, the removal of U from pH adjusted mine water was increasingly efficient approaching 1:200 (Figure 14). At 1:200 (0.5 g biomass/100 mL mine water) the freeze dried fungal biomass removed ≥95% of the U in solution (Figures 7 and 8). Starting with pH 4 mine water, where the U concentration was between 130-140 ppm, this ≥95% value is calculated based on the fact that approximately 5 ppm U in the mine water was remaining following contact with the biomass. The full extent of biosorption of U was complete with 15 minutes, as evident from the slight increase in U concentration of the loading effluent from 4.6 ppm (15 min.) to 8.6 ppm (60 minutes). Both biomasses tested, the DS 200 and the FS 300 performed equally efficient in this S:L of 1:200.

The 1:500 test resulted in less U being removed from solution (Figure 9). Approximately 50 ppm U was remaining in the mine water following contact with 0.2 g biomass in 100 mL U-mine water. This reduction from 130-140 ppm to 50 ppm represents a maximum of 65% U removal.

At 1:1000, the proposed ideal S:L ratio of 1 gram biomass per liter mine water, 2 different loading assays had different results. One, (Figure 10) removed 60 ppm, while the other removed 80 ppm (Table 11). With the different sources of error present in testing, sampling and assaying, these values could be considered similar, although statistically unverified. The amount of U removed from the 130-140 ppm solutions would, depending on the values calculated, result in between 62% to 50% removal.

Elution with carbonate of the 1:1000 (S:L) gave anomalous results (Table 11). With ≤80 ppm U loaded from the mine water, the carbonate eluant was assayed as having 105 ppm U, more U than was theoretically loaded. This discrepancy was not present in the 1:2000 test work.

At a S:L of 1:2000 (0.05 g/100 mL) the loading capacity was between 40-50 mg U per 0.5 g biomass or 80-100 mg U per gram biomass. These values were consistent with all the biomasses tested (at this S:L) whether the biomass was FS 200, FS 300 or DS 300 (Figures 11-13). Elution with 5% carbonate was done only on the DS 300 biomass (Table 11). With 45-55 ppm U removed from the 130-140 ppm U-mine water, a total of 56 ppm U was assayed in the eluant.

The hydrodynamic properties of the biomass were noted during the loading and elution testwork. All the biomass tested, when wetted, did not float, but rather remained submerged. This property will contribute favourably to any hydrometallurgical processing scheme that the biomass can be potentially incorporated.

DISCUSSION

The success of obtaining sufficient quantities of biomass grown on spent brew grain requires some explanatory notes. The process by which Connor's Brewery makes beer is one whereby part of the grain, which is used in the wort preparation, is such that 100% of the sprouting has not taken place. This results in the spent grain being richer in carbohydrate values than that used in the larger breweries. This factor is apparently only present in the microbrewery operations where they themselves malt

the barley for wort preparation. From the harvest of about 4 g dry wt./L SBG medium , it would appear that soluble metabolizable carbohydrate were present in at least 10 g/L concentrations (at ≈50% utilization). The use of spent brew grain from microbreweries such as Connors, therefore, is probably advantageous in the preparation of a viable growth medium.

Harvesting the biomass before the decline of growth from the logarithmic phase has been investigated in other work. Scharer and Byerley (1989) noted that prolonged incubation after the cessation of growth resulted in the reduction of the capsular polysaccharide, considered by these researchers as qualitatively important in U-adsorption. Treen-Sears et al. (1984) observed decreased U-uptake with late harvested biomass. These observations prompted the timely harvest of biomass in this investigation, but was not tested for contrary findings.

Loading of U from the mine water to the biomass displayed a maximum of 62% efficiency with a 1:1000 S:L ratio. Although less than the ideal 180 mg U/g biomass, the fact that actual mine water was used in the present investigation suggests maximum utilization. In determining U-uptake potentials of 180 mg/g biomass, Tzezos and Volesky (1981,1982) used synthetic solutions of uranyl nitrate as did Treen-Sears et al. (1984) and Tobin et al. (1984), both without the same results but both with high U biosorption. In work by Scharer and Byerley (1989), it was determined that biosorption capabilities in process solutions were substantially less (50 mg U/g biomass) than in synthetic solutions (121 mg/g) of similar uranium and sulphate content. Recently, Tsezos et al, (1989) has shown non-immobilized Rhizopus biomass to uptake about 200 mg U/g biomass from a synthetic uranyl nitrate solution of 500 mg U/L, while immobilized biomass when contacted with process solutions was capable of about 50 mg U/g biomass. As the results of this investigation were realized with process solutions, the possibility does exist that maximum U-uptake was determined.

4. POTENTIAL COSTS

The cost of producing the Rhizopus arrhizus biomass in spent brew grain is compared against a commercially available nutrient growth medium, Potato Dextrose Broth. Indications from the brewmaster at Connor's Brewery are that they give the waste free of charge to a pig farmer, so long as there are no delays in pick-up on a demand basis. The SBG could therefore be considered as costing nothing except for the potential transportation costs. To produce 1 gram of dry weight biomass from the PDB, approximately 50 g of dry medium are required; at $80/lb, this calculates to $8.50 per g biomass. Depending on the streamlining of mass production of SBG extract, the energy costs and manpower could ultimately approximate any costs associated with PDB preparation.

In similar work done for DSS, Tsezos (1989) has produced R. arrhizus biomass in approximately the same quantities using a proprietary nutrient medium. The major difference between the two methods (proprietary vs SBG), is the growth time of 40-50 hours (72 hrs., prep.). This would have the potential of introducing a cost reduction in biomass preparation of about 2/3.

Unavoidable expenses incurred for both types of biomass production would include the electrical costs of autoclaving, agitation, freeze drying as well as any manpower needed in the biomass production. If these expenses are controlled with efficiency, then the difference in costs may only be the transportation of the SBG vs. the price of the PDB. With these parameters outlined, there could be a potential savings of up to $8.00 per gram useable biomass.

5. CONCLUSION AND RECOMMENDATIONS

The testwork undertaken by ORTECH International has proven that the U biosorbing fungus Rhizopus arrhizus can be grown in sufficient quantities utilizing food industry waste as a growth medium. Specifically, spent brew grain (Connor's Brewery, Toronto) obtained immediately following wort preparation, was the food waste which allowed for the consistent production of about 20 grams wet weight biomass per liter growth medium. Optimal growth conditions have been determined as being:

1. growth medium prepared from an extract of the spent brew grain at 400 g SBG/L water.
2. an inoculum concentration resulting in 2500 spores/mL growth medium.
3. a high rate of agitation; in this work, 300 rpm on a gyrotary shaker.

The fungus has shown full growth to be complete within 50 hours and a corresponding end pH of between 3.0 and 3.25 is recommended for harvesting. Removal of U from mine water solutions was optimal with a solid to liquid ratio of 1:200, effectively removing about 95% of the U. At the ideal lower S:L of 1:1000 there was between 50% and 62% U removal, not an unreasonable amount when process solutions are used. Carbonate elution of the assayed loaded biomass was ≥100% effective at S:L ratios of 1:1000 and 1:2000.

The production of this food waste grown biomass has the potential to have economic advantages over proprietary media. A savings of up to $8.00 per g useable biomass has been predicted, resulting in the necessary cost-efficiency demanded by this project.

The work completed in this investigation suggests a number of ways in which the process could be developed further.

The incorporation of this biomass, as harvested in spent brew grain medium, into an immobilization scheme already developed. This could allow for any modifications necessary as well as give an idea of actual processing considerations. The use of just the cell walls of the fungal isolate may also increase the loading capacity per gram of the immobilized biomass.

Because of the non-selective nature of metal binding by Rhizopus arrhizus biomass, this biomass should be tested for the removal of metals from other industrial process solutions. The potential exists out of this technology to aid in a variety of effluent treatment.

Further optimization of the growth of this Rhizopus arrhizus may be realized by studying the effects of temperature, pH of the process solutions, and alternative brewery - supplied waste grain.

6. CONTRACT PLAN, WORK PROGRAM AND CASH FLOW

Extensions to this project were given in August and September 1989 to October 31,1989. This allowed for absences due to vacation and business meetings. Task 4, designated as ongoing and with a planned cost, had costs necessarily spread over a long term period and as such were absorbed into the cost of the other tasks. Inspite of the changes in scheduling, the project's tasks were completed in accordance to the overall financial and contract planning, as recorded in the accompanying Table 12.

Supply and Services Canada | Approvisionnements et Services Canada

DSS-MAS 9143-3 (4/81)

CONTRACT PLAN AND REPORT FORM – FORMULE DE PROJET DE CONTRAT ET DE RAPPORT

CONTRACT TITLE – TITRE DU CONTRAT

CONTRACTOR – ENTREPRENEUR

FILE NO. – N° DU DOSSIER	SERIAL NO. – N° DE SÉRIE
41-11111	DSS 23440-8-9: 98 SQ

TASK DESCRIPTION DESCRIPTION DE LA TÂCHE	TASK DURATION IN MONTHS – DURÉE DE LA TÂCHE EN MOIS												TASK COST – COÛT DE LA TÂCHE		
	1 Jan.	2 Feb.	3 Mar.	4 April	5. May	6 June	7 July	8 Aug.	9 Sept.	10Oct.	11	12	PLANNED PRÉVU À L'ORIGINE	ACTUAL PRÉSENTEMENT	E.T.C. À VENIR
Material Procurement															
Canvassing (1)													2K	1315.86	Feb. 1/89
Actual Receipt (2)													5K	2474.15	Mar. 1/89
Chemical Analysis (3)													2K	2000.00	Apr. 1/89
Fungus Maintenance (4)													3K	Ongoing	Ongoing
Medium Design and Formulation (5)													8K	9641.34	May 31/89
Growth in Medium (6)													5K	5975.55	May 31/89
Optimization (7)													5K	9848.08 Ongoing	Sept. 30/89
Harvesting Processing (8)													5K	4845.51	Oct. 31/89
Loading/Elution (9)													5K	4845.51	Octo. 31/89
PLANNED COST BY MONTH COÛT PRÉVU POUR CHAQUE MOIS	2K	5K	10K	15K	8K								PLAN 40946.00		
ACT. PRESENT. / E.T.C. BY MONTH PRÉVISION POUR CH. MOIS	1315.86	2474.15	3666.13	7588.51	5975.55	6497.80	1724.14	1626.14	7065.16	2625.86				ACTUAL COÛTS RÉELS 40946.00	E.T.C. À VENIR Oct. 31/89

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TABLES

Table 1
Survey of industrial food producers and the waste
produced in Southern Ontario

PRODUCERS	RESULT
Freshway Products	no waste
McCarthy Milling	no waste
Connors Brewery	spent grain available
St. Lawrence Corn Starch	all waste to alcohol production
Imperial Flavors Inc.	no waste
GayLea (Yogurt)	no waste
Campbell's Mushroom Farm	hay, mushroom, compost
Campbell's (Chatham)	seasonal waste
Canada Packers Dairy Div.	no waste
General Mills	difficult
Canada Malting	waste water in Thunder Bay
Reid Milling	no waste
Robin Hood Milling (Milton)	no waste
Maple Leaf Mills Rendering	no waste
Nabisco	cereal rejects
Region of Peel	organic sludge matter
Ontario Food Terminal	variety, seasonal
E.D. Smith	seasonal
Heinz	seasonal
Omstead Foods (Wheatly)	fish, onions constantly
Heritage Farms (Bramalea)	milk waste, pig slop.
Hostess Foods (Cambridge)	chip waste, corn screenings, potato mash
Nestles	no waste
Region of Halton	landfilled
Village of Hensall	grain screenings
Amstel Brewery	no waste
City of Toronto	complicated
Protein Foods (Paris)	poultry waste
Victory Soya	soap stock
Pillsbury	corn husks, seasonal

Table 2
Industrial food producers agreeing
to supply food waste products

Producer and food waste obtained:		
Village of Hensall	1)	bean dirt
	2)	grain screenings
Omstead Foods	3)	fish
	4)	onions
Nabisco Foods	5)	cereal rejects (Shredded Wheat shreddies mixed)
Hostess Foods	6)	potato mash
	7)	plain potato chips
	8)	corn mash
Heritage Farms	9)	dairy vitamin beveridge
	10)	pig slop
Campbell Mushroom Farm	11)	mushrooms
	12)	compost
Protein Foods	13)	chicken waste
Connors Brewery	14)	spent grain

Table 3
Composition of samples submitted for
TOC, TKN and TP analysis

Sample #	Description	
UF-01	H_2O/bean dirt	(3/1)*
UF-02	H2O/onions	(1/1)
UF-03	H2O/cereal	(4/1)
UF-04	H2O.fish	(1/1)
UF-05	H2O/grain	(10/1)
UF-06	H2O/potato	(1/3)
UF-07	H2O/potato chips	(2/1)
UF-08	H2O/corn	(2.5/1)
UF-09	H2O/brewery grain	(1/1)
UF-10	H2O/mushrooms	(1.5/2)
UF-11	H2O/mushroom compost	(2/3)
UF-12	undiluted vitamin beveridge	
UF-13	undiluted chicken waste water	
UF-16	pig slop undiluted	

* (3/1) parts H_2O/parts waste

Table 4
Nitrogen, Carbon and Phosphorous
Values of Food Waste Samples

	TKN (ppm)		TOC (%)		Total P (ppm)	
SAMPLE	original suspension	after dilution calculation	original suspension	after dilution calculation	original suspension	after dilutio calculation
Bean	4830	19320	7.81	31.24	712	2848
Onion	570	1140	1.12	2.24	167	334
Cereal	3590	17950	7.78	38.90	639	3195
Fish	8020	16040	6.84	13.68	1950	3900
Grain	730	8030	3.80	41.80	136	1496
Potato	2090	2786	3.68	4.90	250	333
Potato Chip	3260	9780	16.50	49.50	459	1377
Corn	2460	8610	9.07	31.70	747	2615
Brew Grain	4620	9240	5.71	11.42	818	1636
Mushroom	1950	3461	1.92	3.36	356	623
Mush. Comp.	5430	9050	6.40	10.67	1550	2583
Vitamin Bev.	5200	5200	15.00	15.00	423	423
Chicken w.w.	570	570	3.92	3.92	127	127
Bean	5540	22160	7.36	29.44	791	3164
Onion	500	1000	1.22	2.44	166	332
Pig Slop	6550	6550	9.02	9.02	747	747

TKN: Total Kjeldahl Nitrogen
TOC: Total Organic Carbon
TOTAL P: Total Phosphorous

Table 5

Raw Blended Food Waste Growth Characteristics

Food-waste medium	Day 3 growth	Day 6 growth
Bean dirt	thick growth on flask-walls easily dislodged in the suspension suspension	same as day 3
Cereal	thin mycelium layer in upper flask	thick mycelial growth in upper flask
Fish	thick ring of growth at swirl point	same as day 3
Potato	thin growth ring at swirl	more growth in upper flask
Corn	thick growth at swirl	same as day 3
Spent Brew Grain	thin growth ring, upper flask growth	same as day 3
Mushrooms	very thin mycelium in upper flask	very poor to no growth
Vitamin Beverage	no growth visible	no growth
Pig Slop	not growth visible	no growth
Fish/Grain Screening	ring of mycelium at swirl	same as day 3
Fish/Potato	thick growth ring at swirl, upper flask growth	same as day 3
Fish/Potato chips	thin layer in upper flask	same as day 3
Potato/corn	growth ring at swirl, upper flask growth	same as day 3
Chips/Corn/ Vitamin Bev/Pig slop	very thin growth in upper flask	same as day 3
Fish/Potato/ Mushroom	thick growth ring in upper flask	same as day 3
Potato Dextrose Broth (Shaker)	good growth in suspension	same as day 3
PDB (Stationary)	good growth in suspension	same as day 3

Growth conditions: 23°C 50% RH Gyrotary shaker at ≈200 rpm

day 1: no growth observed in any preparation

Table 6
Stationary Food Waste Growth Characteristics

Waste	Day 5 Characteristics
Cereal	thick lawn of mycelium on medium surface and flask walls, minor sporulation
Potato	same as above, more advanced sporulation
Corn	thin lawn of mycelium, some sporulation
Fish	same as cereal
Bean	same as cereal, sporulation on side walls
23°C 50% RH	Stationary Conditions Raw Blended Food Waste

Table 7

Extracts made were based on results of food waste growth screening tests as listed in Table 2. Good growth on the raw blender-ground food waste was the only parameter for choosing potential extracts.

Raw Material	Raw Material (g)	Vol. of H_2O (mL)	Boiling Time (min)	Filtration	Final Vol. (mL)
Corn	160	1000	60	vacuum, muslin	510
Fish	200	1000	60	vacuum,muslin	670
Cereal	50	1000	60	(125 lb) pressure, muslin	600
Bean Dirt	40	1000	10	(125 lb) pressure, muslin	740
Grain Screening	200	1000	60	(50 lb) pressure, muslin	320
Spent Brew Grain	200	1000	60	(50 lb) pressure, muslin	540
Potato Chip	100	1000	30	(125 lb) pressure, muslin	490
Potato	200	1000	30	(50 lb) pressure, muslin	770

Double layer of fine muslin cloth was used as the filter medium. The texture of the boiled raw material determined the amount of pressure during filtration; course material such as grain screenings would allow only 50 lb. of pressure to build up, of the 125 lb. maximum. Tap water was used as the aqueous phase.

Table 8

Extract Growth Characteristcs

Bean (DIL)	Dark mycelium evident, less than PDB
Bean (FS)	Dark mycelium evident, less than PDB
Grain Screenings (DIL)	very little growth, mostly at swirl ring
Grain Screenings (FS)	very little growth, mostly at swirl ring
Cereal (DIL)	good growth, mostly at swirl ring
Cereal (FS)	good growth, mostly at swirl ring
Corn (DIL)	about 1/2 as much as (FS) corn
Corn (FS)	very good growth clump, and swirl growth
Fish (DIL)	thin ring growth only
Fish (FS)	thin ring growth only
PDB	good clump of mycelium in suspension

Growth conditions as in Table 4

DIL: recovered extract volume diluted in 1 L

FS: recovered extract volume undiluted

Table 9
Gravimetric Determination of Rhizopus arrhizus
Grown on Different Sources of Food Waste Extract

FOOD WASTE EXTRACT	BIOMASS WEIGHT (g)/100mL MEDIA
CORN (FS)	0.23, -0.16
CORN (DIL)	0.11
GRAIN SCREENING (FS)	0.02
GRAIN SCREENING (DIL)	-0.10
BEAN DIRT (FS)	0.30, 0.01, -0.03
BEAN DIRT (DIL)	0.29
CEREAL (FS)	0.038, 0.14, 0.14
CEREAL (DIL)	0.50
FISH (FS)	0.21
FISH (DIL)	-0.10
POTATO (FS)	-0.11
POTATO CHIP (FS)	-0.04
BREW GRAIN (FS)	0.10
BREW GRAIN (FS)	0.10
BREW GRAIN (FS)	0.11
FISH/GRAIN SCREENINGS	0.18, 0.17, -0.20
POTATO/CORN	-0.01
FISH/POTATO	0.07
POTATO DEXTROSE BROTH	0.18, 0.11, 0.20

(FS) = FULL STRENGTH MEDIUM
(DIL) = DILUTE MEDIUM

GROWTH CONDITIONS: 23°C, 50% RH, ORBITAL SHAKING 200 rpm

Development of an Economic Large Scale Growth Medium for Rhizopus arrhizus for Department of Supply and Services

Table 10

Maximum Chemical Values of Extracts

	FOOD WASTE								
	Spent Brew Grain		Cereal	Corn	Fish	Bean Dirt	Grain Screens	Potato	Potato Chip
	FS	2X							
Amount Per Extract (g/L)	200	400	50	160	200	40	200	200	100
TOC* (g)	22.840	45.680	19.450	50.720	27.360	12.500	83.600	9.800	49.500
TKN* (g)	1.848	3.696	0.897	1.378	3.208	0.773	1.606	0.557	0.978
TP* (g)	0.372	0.744	0.160	0.418	0.780	0.114	0.299	0.066	0.137
S.G.	1.002	1.005	1.003	1.003	1.004	1.002	1.005	1.006	1.007
g sucrose**/L	10.0	18.0	12.5	12.5	15.0	10.0	17.8	20.1	23.0

* Maximum values in the raw food waste.
** For comparison purposes only.
TOC: Total organic Carbon
TKN: Total Kjeldahl Nitrogen
TP: Total Phosphorous
S.G.: Specific Gravity (tap water = 1.000)
F.S.: Full Strength; 2x-Double Strength

Table 11

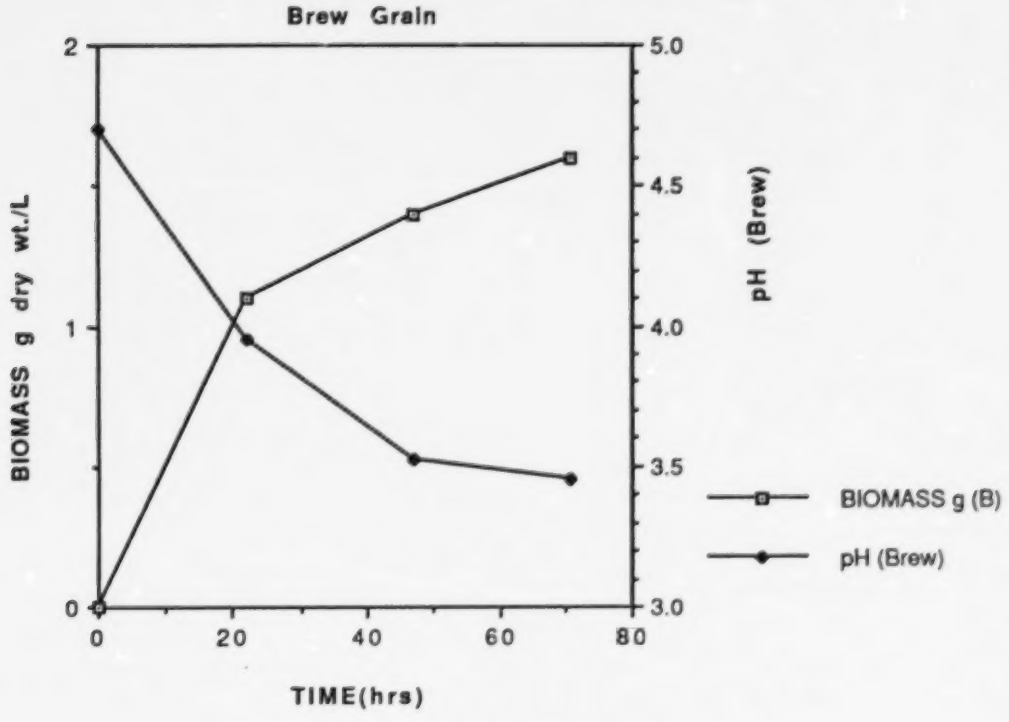
Effect of Carbonate (Na_2CO_3, 5%) on U-loaded biomass

S:L	U (ppm) before biomass contact	U (ppm) after biomass contact	U (ppm) uptake	U (ppm) after carbonate elution
1:1000	130	86	54	56
1:2000	130	59	81	105

FIGURES

FIGURE 1

Comparison of Growth of Rhizopus arrhizus in Spent Brew Grain and Cereal Waste Extract



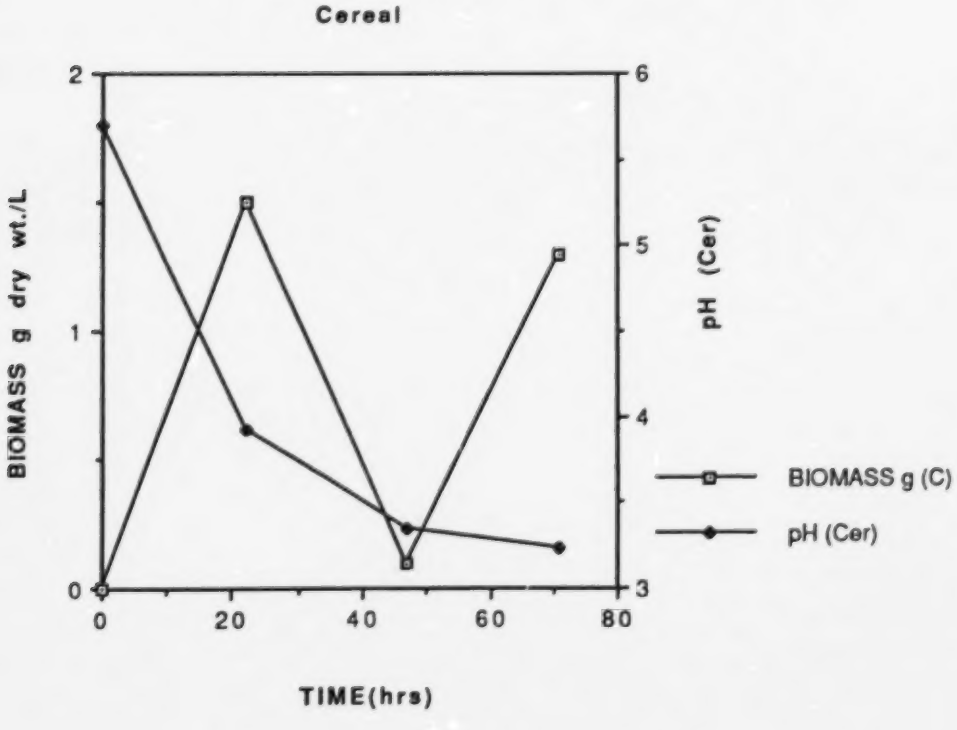
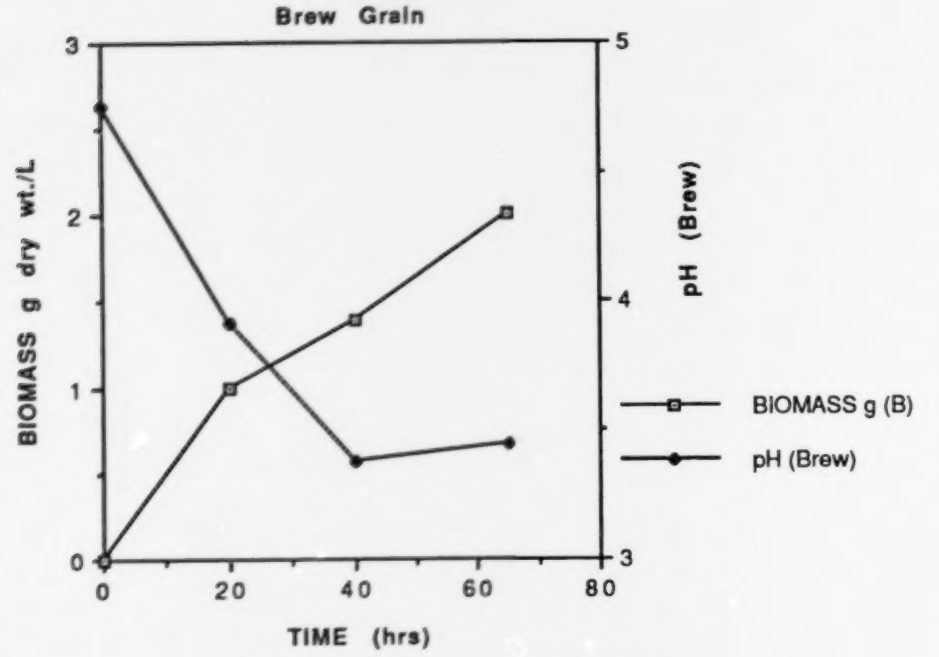


FIGURE 2
Comparison of Growth in Cereal and Spent Brew Grain



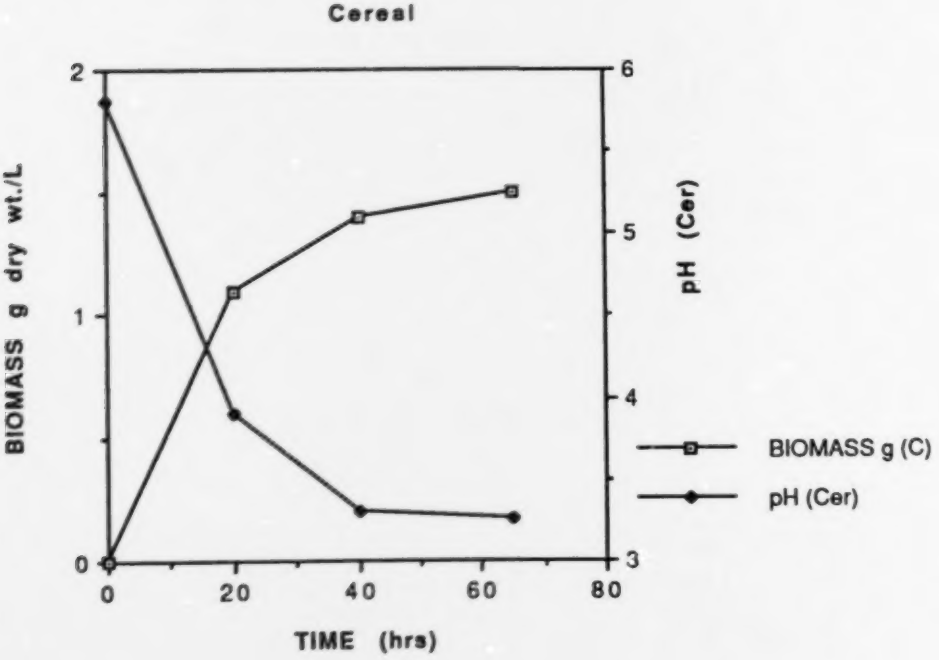
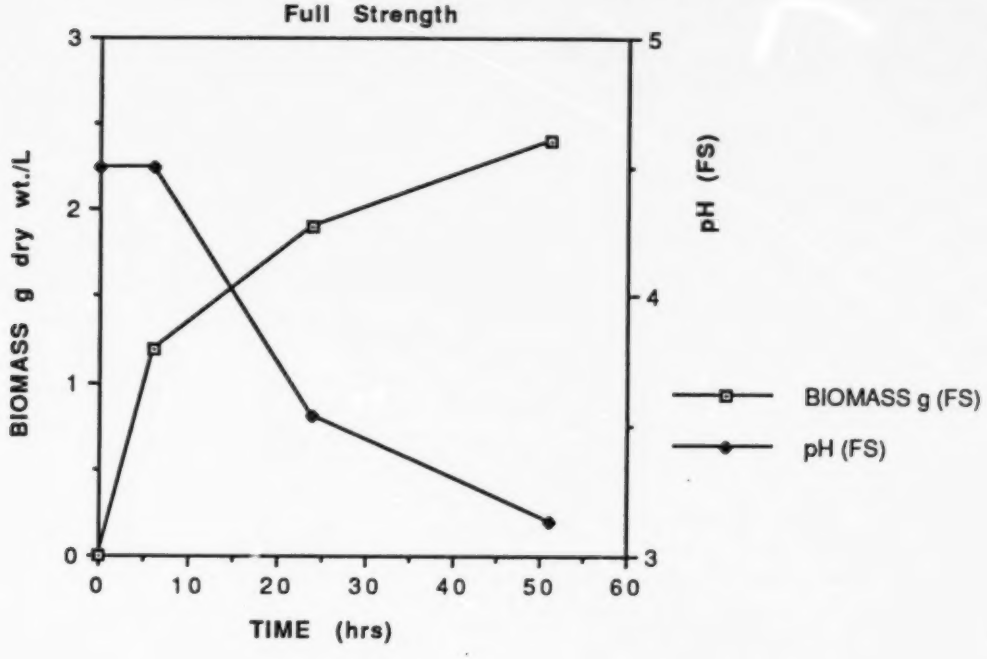


FIGURE 3

Comparison of Growth in Full Strength (200 g/L)

and Double Strength Spent Brew Grain Extract Agitated at 200 rpm



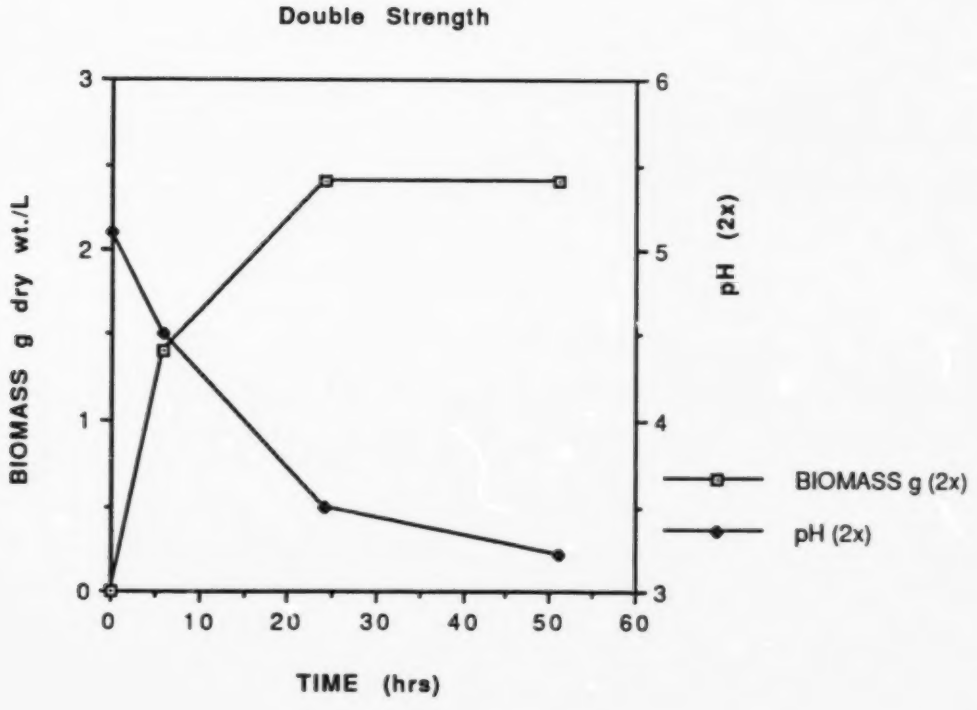
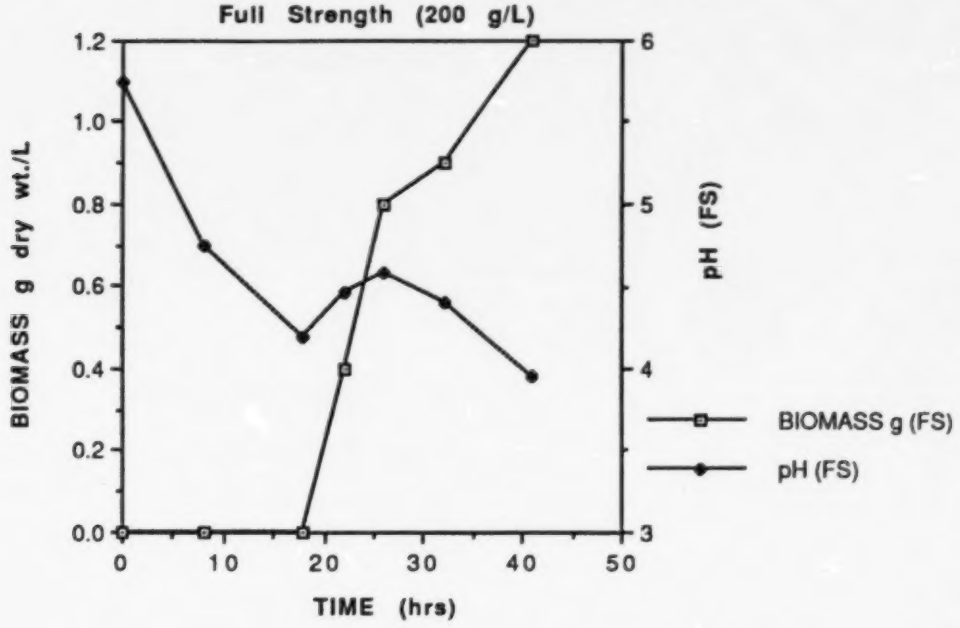


FIGURE 4

Comparison of Growth of Rhizopus arrhizus in Full Strength and Double Strength Agitated at 200 rpm



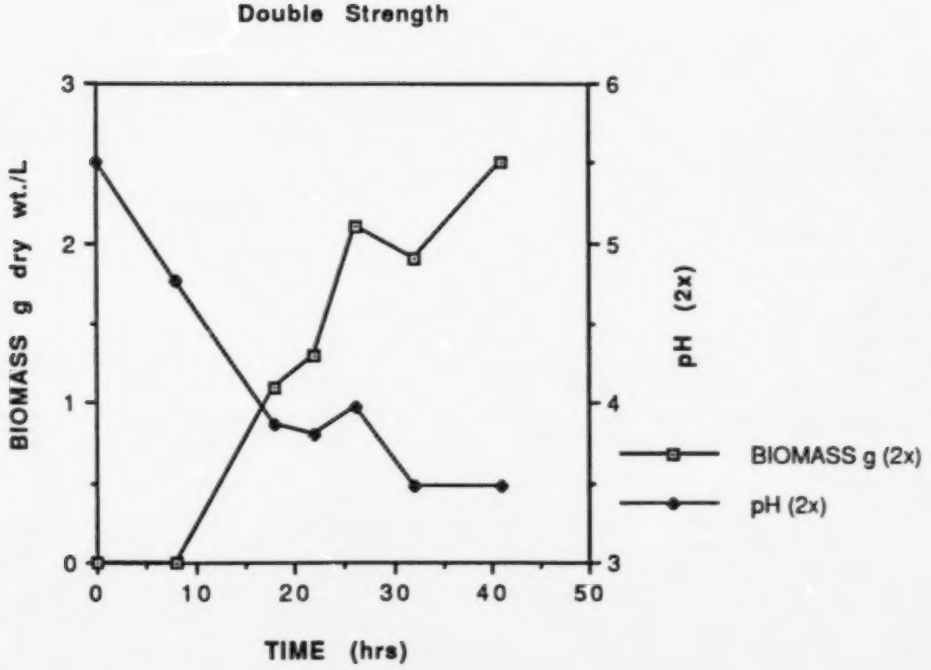
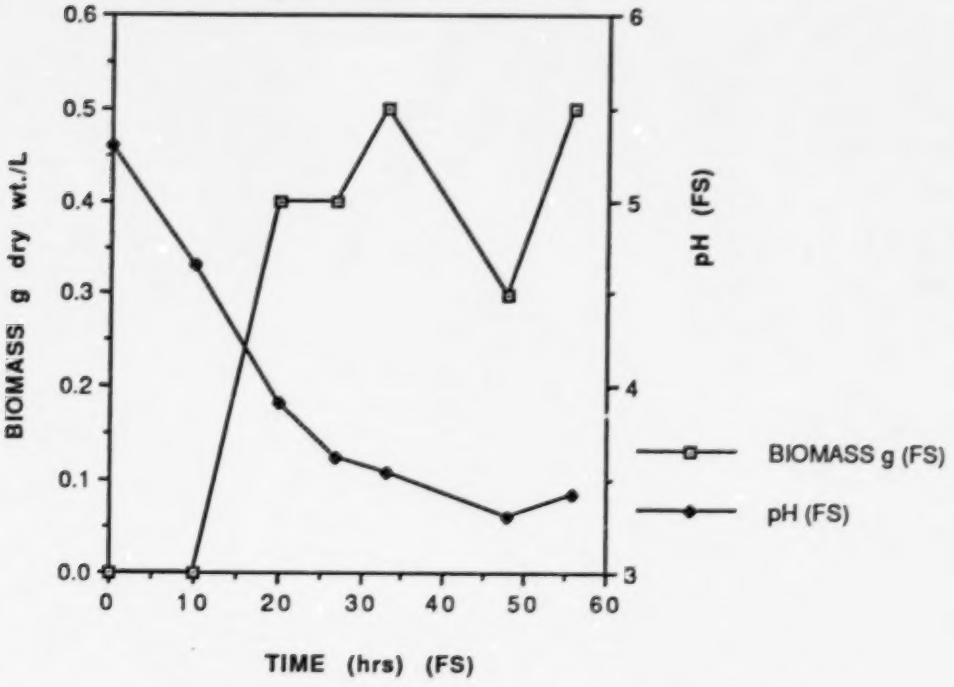


FIGURE 5

Comparison of Growth of Rhizopus arrhizus in Full Strength and Double Strength Agitated at 300 rpm

Full Strength at 300 rpm shaking



Double Strength at 300 rpm

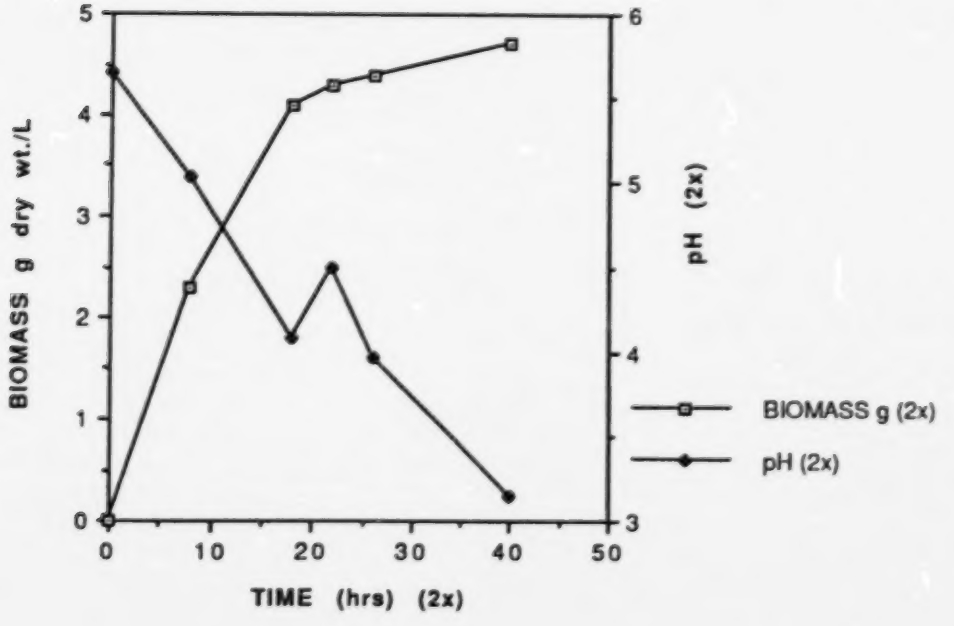


FIGURE 6

Growth of Rhizopus arrhizus in spent Brew Grain Extract (400 g/L) at 300 rpm shaking

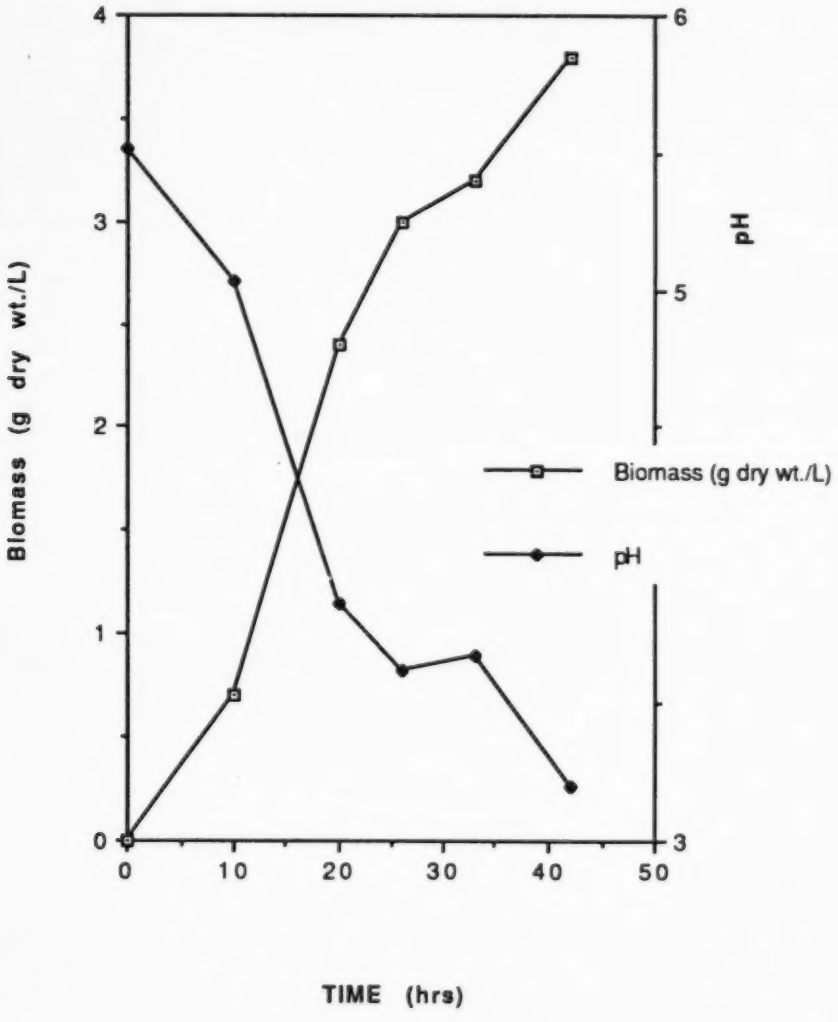


FIGURE 7

Removal of U from Denison mine water by Rhizopus arrhizus biomass (FS 300) in a solid to liquid ratio 1:200

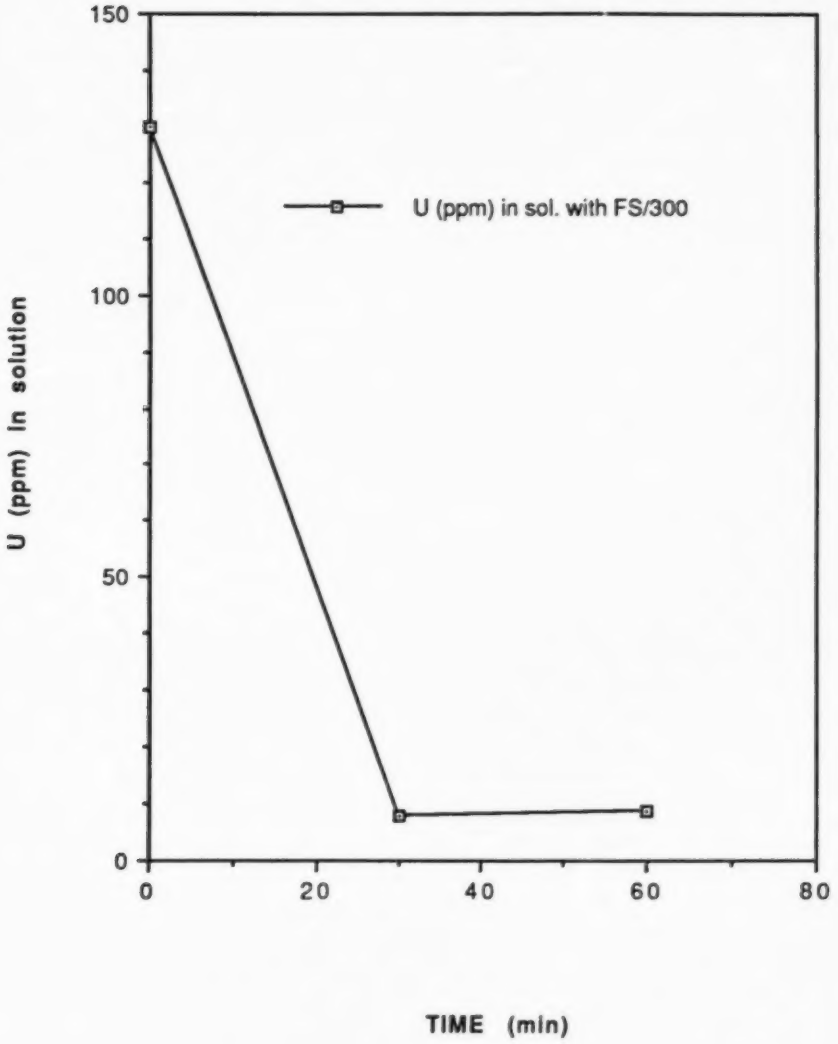


FIGURE 8

Removal of U from Denison mine water by Rhizopus arrhizus biomass (DS 200) in a solid to liquid ratio 1:200

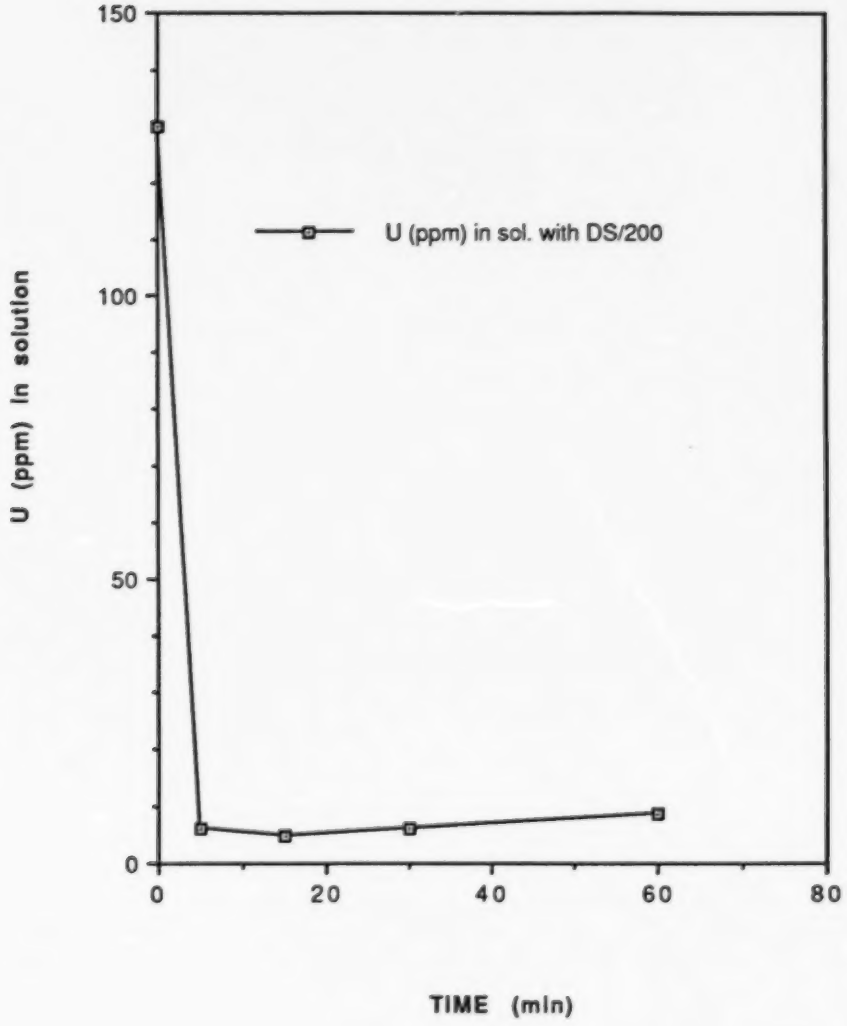


FIGURE 9

Removal of uranium from Denison mine water by Rhizopus arrhizus biomass (DS 300) at a solid to liquid ratio of (1:500)

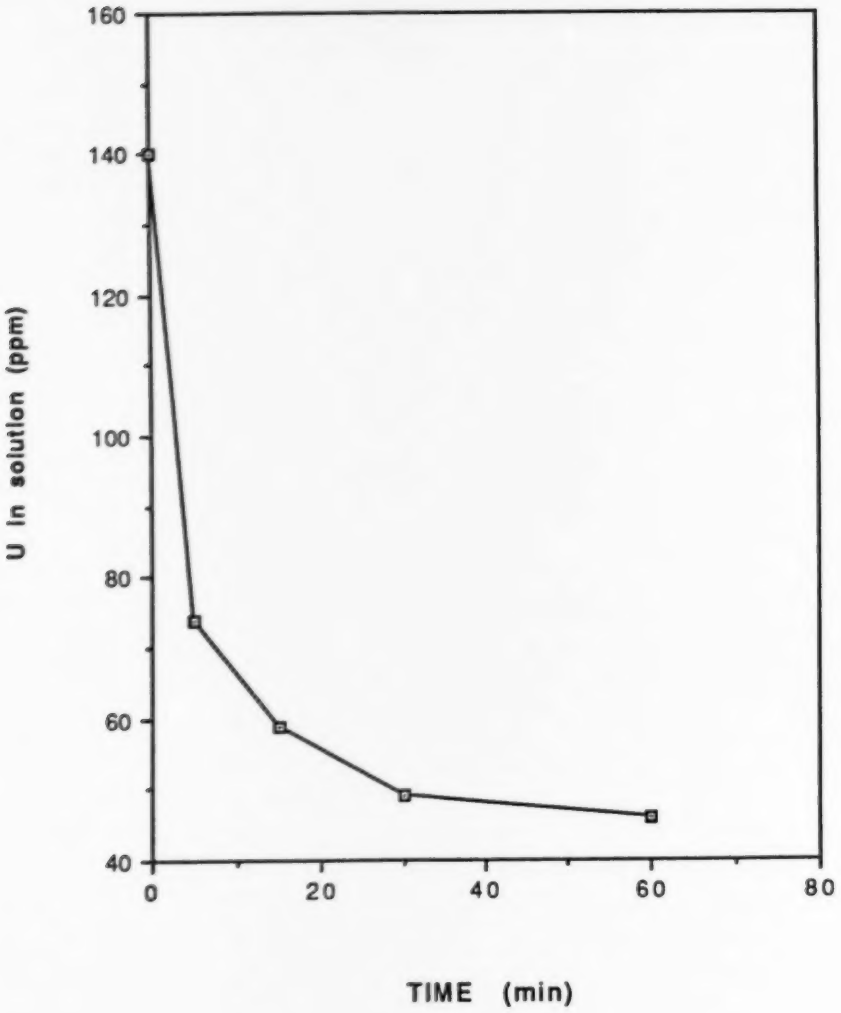


FIGURE 10

Removal of uranium from Denison mine water

by Rhizopus arrhizus biomass at a solid to liquid ratio of (DS 1:1000)

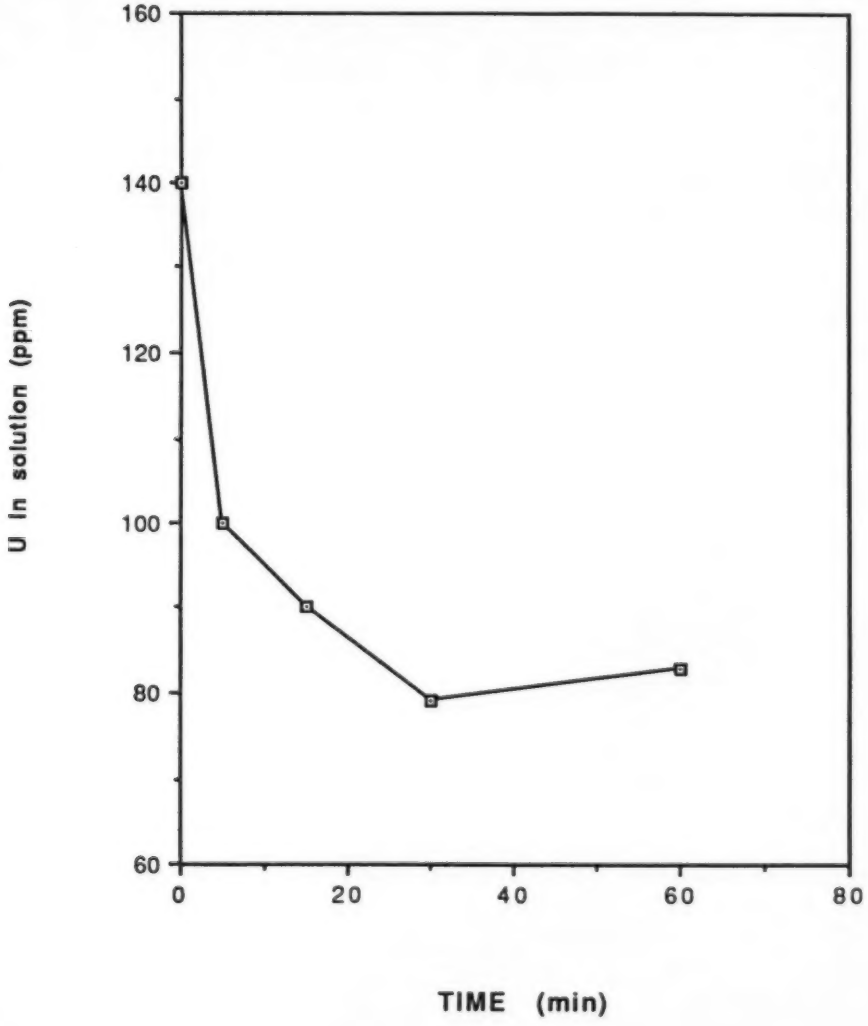


FIGURE 11

Removal of U from Denison mine water

by Rhizopus arrhizus biomass (DS 300)

in a solid and liquid ratio 1:2000

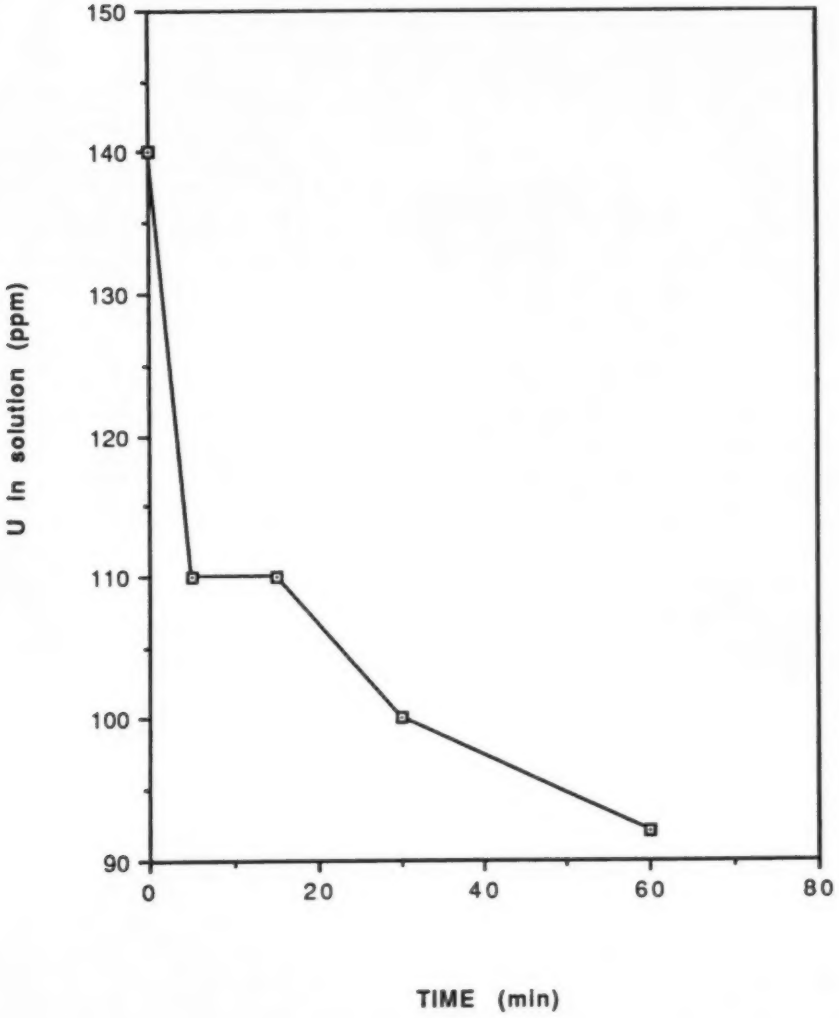


FIGURE 12

Removal of U from Denison mine water

by Rhizopus arrhizus biomass (FS 200)

in a solid and liquid ratio 1:2000

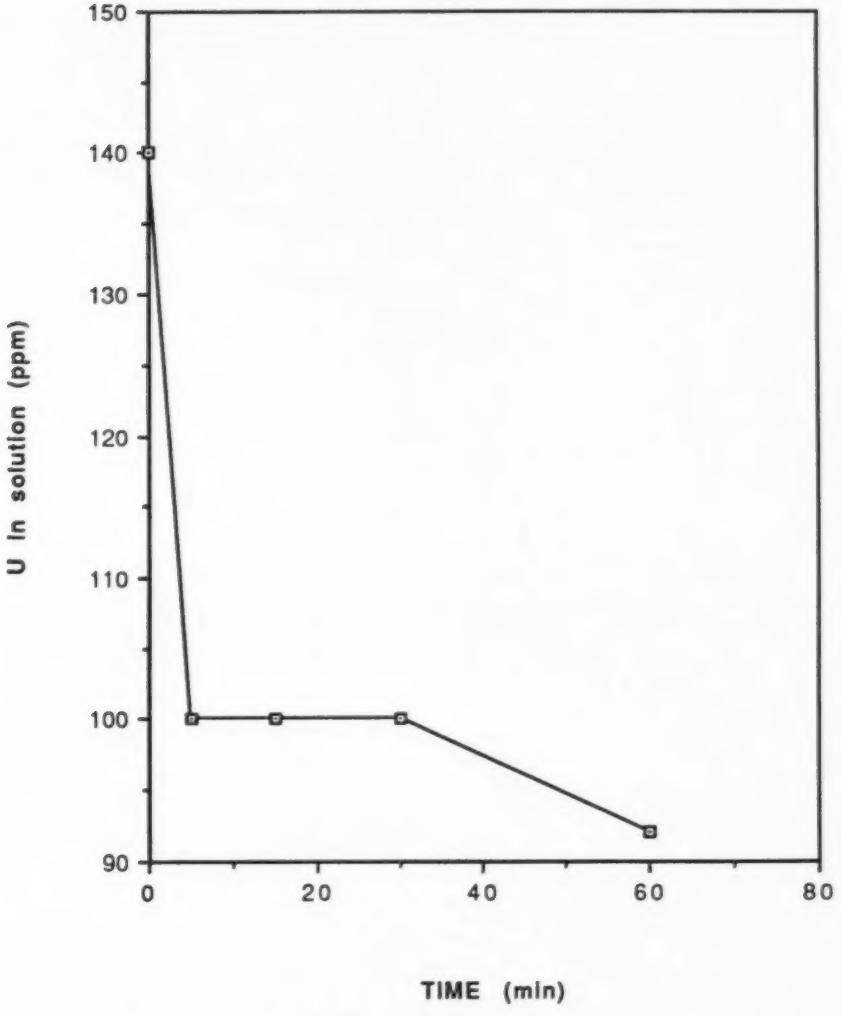


FIGURE 13

Removal of U from Denison mine water

by Rhizopus arrhizus biomass (FS 300)

in a solid and liquid ratio 1:2000

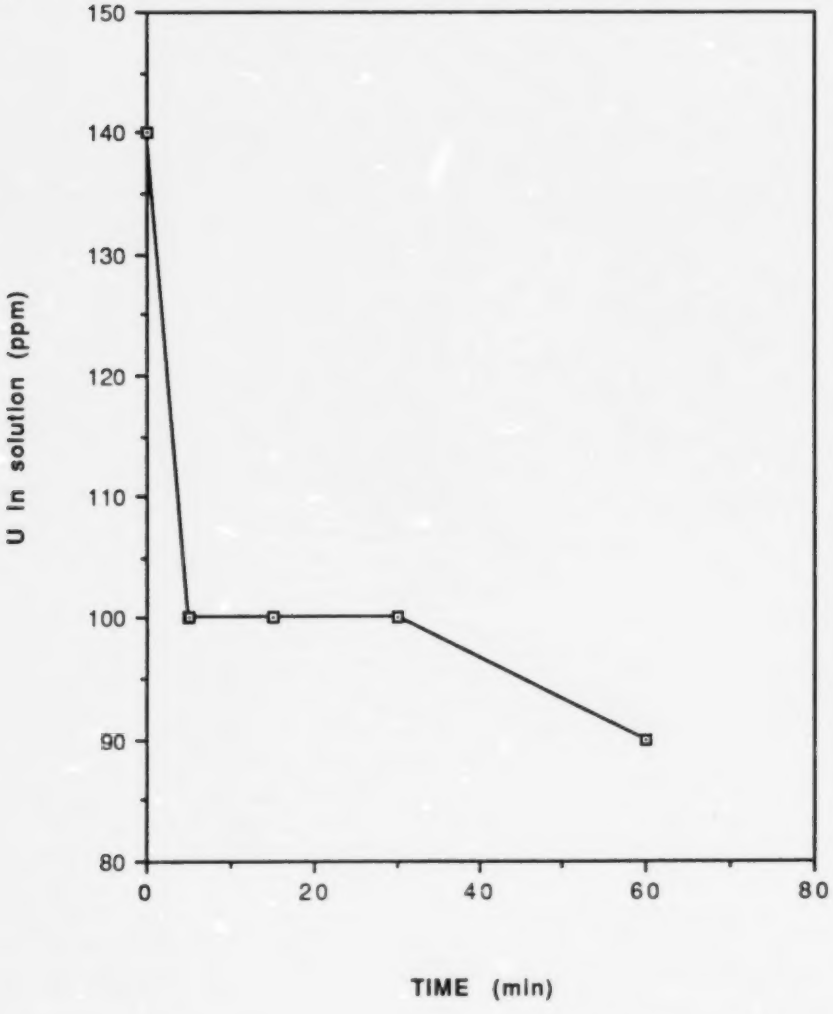


FIGURE 14

Effect of Solid to Liquid Ratio on U-Biosorption

initial [U] = 140 ppm